

CLAIMS

1. An in situ bioreactor adapted for systemic delivery of bioactive agents, comprising a first nucleic acid molecule encoding a cell growth stimulating agent, a second nucleic acid molecule encoding a bioactive agent, and a biocompatible substance capable of cellular infiltration.

2. The bioreactor of claim 1, wherein the cell growth stimulating agent is selected from the group consisting of: a transcription factor, a chemotactic factor, an angiogenic factor, an antisense molecule, a ribozyme, an anti-apoptotic molecule, a growth factor, a cytokine, an extracellular matrix molecule, a cell adhesion protein, a cell retention agent, and a cell surface receptor.

3. The bioreactor of claim 2, wherein the first nucleic acid molecule encodes a growth factor or cytokine.

4. The bioreactor of claim 3, wherein the growth factor is selected from the group consisting of: transforming growth factor (TGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF), insulin like growth factor (IGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF), colony stimulating factor (CSF), angiopoietin, interleukin and bone morphogenic factor (BMP) family members.

5. The bioreactor of claim 4, wherein the growth factor comprises one or more PDGF family members.

6. The bioreactor of claim 5, wherein the growth factor is PDGF-B.

7. The bioreactor of claim 4, wherein the growth factor is HGF.

8. The bioreactor of claim 4, wherein the growth factor comprises one or more FGF family members.

9. The bioreactor of claim 8, wherein the growth factor is FGF-2.

10. The bioreactor of claim 9, wherein the FGF-2 is a mutated FGF-2.

11. The bioreactor of claim 8, wherein the growth factor is FGF6.

12. The bioreactor of claim 4, wherein the growth factor is one or more TGF family members.

13. The bioreactor of claim 12, wherein the growth factor is selected from the group consisting of TGF- β 1, TGF- β 2, and TGF- β 3.

14. The bioreactor of claim 2, wherein the cell growth stimulating agent is an antisense molecule.

15. The bioreactor of claim 2, wherein the cell growth stimulating agent is a ribozyme molecule.

16. The bioreactor of claim 2, wherein the cell growth stimulating agent is an anti-apoptotic agent.

17. The bioreactor of claim 16, wherein the anti-apoptotic agent is Bcl-2.

18. The bioreactor of claim 16, wherein the anti-apoptotic agent is Bcl-xL.

19. The bioreactor of claim 16, wherein the anti-apoptotic agent is A20.

20. The bioreactor of claim 2, wherein the tissue growth stimulating factor is a transcription factor.

21. The bioreactor of claim 20, wherein the transcription factor is an activator or a repressor.

22. The bioreactor of claim 21, wherein the transcription factor is selected from the group consisting of: NF- κ B, E2F, DP1, AP-1, AP-2, myc, p53, Sp1, NFAT, CBP, C/EBP, and nuclear hormone receptor family members.

23. The bioreactor of claim 1, wherein the bioreactor further comprises a cell retention agent.

24. The bioreactor of claim 1, wherein the bioreactor further comprises a nucleic acid encoding a cell retention agent.

25. The bioreactor of claims 23 or 24, wherein the cell retention agent is selected from the group consisting of: macrophage migration inhibitory factor (MIF), extracellular matrix molecules, and cell adhesion molecules.

26. The bioreactor of claim 1, wherein the second nucleic acid molecule encodes a hormone.

27. The bioreactor of claim 26, wherein the hormone is selected from the group consisting of: growth hormone, insulin, atrial natriuretic peptide (ANP), luteinizing hormone, follicle-stimulating hormone, releasing hormones, inhibin, relaxin, activin, and follitropin.

28. The bioreactor of claim 27, wherein the hormone is insulin.

29. The bioreactor of claim 1, wherein the second nucleic acid molecule encodes a bioactive agent selected from the group consisting of: Factor V (FV), Factor VII (FVII), Factor VIII (FVIII), Factor IX (FIX), Factor X, (FX), Factor XI (FXI), Factor XIII (FXIII), erythropoietin (EPO), growth hormone (GH), adenosine deaminase, thrombopoietin, purine nucleoside phosphorylase (PNP), Protein C, Protein S, an interleukin, an interferon, a globin, an antibody, and an antibody fragment.

30. The bioreactor of claim 1, wherein the second nucleic acid molecule encodes a fibrinolytic agent.

31. The bioreactor of claim 30, wherein the fibrinolytic agent is selected from the group consisting of: tissue plasminogen activator, plasminogen, plasmin, urokinase, and streptokinase.

32. The bioreactor of claim 1, wherein the second nucleic acid molecule encodes an anticoagulant.

33. The bioreactor of claim 32, wherein the anticoagulant is selected from the group consisting of: thrombomodulin, Protein C activating agents, Protein C, and antithrombin.

34. The bioreactor of claim 1, wherein the second nucleic acid encodes a coagulant.

35. The bioreactor of claim 34, wherein the coagulant is selected from the group consisting of: thrombin, fibrinogen, fibrin stabilizing factor, Factor IX, Factor VIII, von Willebrand factor, and Factor X.

36. The bioreactor of claim 35, wherein the anticoagulant is Factor IX.

37. The bioreactor of claim 29, wherein the second nucleic acid molecule encodes FVIII.

38. The bioreactor of claim 29, wherein the second nucleic acid molecule encodes EPO.

39. The bioreactor of claim 1, wherein the first and second nucleic acid molecules are operably linked to promoters.

40. The bioreactor of claim 39, wherein the promoters may be independently selected from group consisting of constitutive, inducible, event specific, and tissue specific promoters.

41. The bioreactor of claim 1, wherein the nucleic acid molecule is in the form of a plasmid, or a recombinant insert in the genome of a virus.

42. The bioreactor of claim 41, wherein the virus is selected from the group consisting of an adenovirus, an adeno-associated virus, and a retrovirus.

43. The bioreactor of claim 42, wherein the virus is an adenovirus.

44. The bioreactor of claim 1, wherein the nucleic acid molecule is associated with a condensing agent.

45. The bioreactor of claim 44, wherein the condensing agent is a polycationic agent.

46. The bioreactor of claim 1, wherein at least one nucleic acid molecule is associated with a cell surface binding moiety.

47. The bioreactor of claim 46, wherein the binding moiety is a polypeptide reactive with a fibroblast growth factor receptor.

48. The bioreactor of claim 46, wherein the polypeptide reactive with an FGF receptor is selected from the group consisting of FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, FGF-15, FGF-16, FGF-17, FGF-18, FGF-19, FGF-20, and FGF-21 or fragments thereof that bind to an FGF receptor.

49. The bioreactor of claim 1, wherein the biocompatible substance is a biological matrix.

50. The bioreactor of claim 49, wherein the biological matrix comprises a polymer.

51. The bioreactor of claim 49, wherein the biological matrix is selected from the group consisting of collagen, purified proteins, purified peptides, polysaccharides, glycosaminoglycans, and extracellular matrix compositions.

52. The bioreactor of claim 49, wherein the biological matrix comprises fibrin.

53. The bioreactor of claim 49, wherein the biological matrix comprises collagen.

54. The bioreactor of claim 53, wherein the collagen is type I collagen.

55. The bioreactor of claim 53, wherein the collagen is type II collagen.

56. The bioreactor of claim 51, wherein the polysaccharides are selected from the group consisting of chitosan, alginate, dextran, hyaluronic acid, and cellulose.

57. The bioreactor of claim 1, wherein the biocompatible matrix is a synthetic matrix.

58. The bioreactor of claim 57, wherein the synthetic matrix comprises a polymer.

59. The bioreactor of claim 58, wherein the polymer is selected from the group consisting of polyesters, polyethers, polyanhydrides, polyalkylcyanoacrylates, polyacrylamides, polyorthoesters, polyphosphazenes, polyvinylacetates, block copolymers, polypropylene, polytetrafluoroethylene (PTFE), and polyurethanes.

60. The bioreactor of claim 58, wherein the polymer comprises lactic acid.

61. The bioreactor of claim 58, wherein the polymer comprises glycolic acid.

62. The bioreactor of claim 58, wherein the polymer is a copolymer.

63. The bioreactor of claim 62, wherein the copolymer comprises lactic acid and glycolic acid (PLGA).

64. The bioreactor of claim 1, wherein the biocompatible substance is biodegradable.

65. The bioreactor of claim 1, wherein the biocompatible substance is non-biodegradable.

66. The bioreactor of claim 65, wherein the non-biodegradable substance comprises a polymer selected from the group consisting of poly(dimethylsiloxane) and poly(ethylene-vinyl acetate).

57. The bioreactor of claim 1, wherein the biocompatible matrix is a synthetic matrix.

67. The bioreactor of claim 1, wherein the biocompatible substance is selected from the group consisting of collagen, metal, hydroxyapatite, bioglass, aluminate, bioceramic materials, hyaluronic acid polymers, alginate, acrylic ester polymer, lactic acid polymer, glycolic acid polymer, lactic acid/glycolic acid polymer, purified proteins, purified peptides, and extracellular matrix compositions.

68. The bioreactor of claim 67, wherein the biocompatible substance is a lactic acid/glycolic acid polymer.

69. The bioreactor of claim 1, wherein the biocompatible substance is associated with an implantable device.

70. The bioreactor of claim 69, wherein the device is selected from the group consisting of: a stent, a catheter, a fiber, a hollow fiber, a patch, and a suture.

71. The bioreactor of claim 69, wherein the device contains expanded polytetrafluoroethylene (ePTFE) or Dacron.

72. A method adapted for systemic delivery of a protein from a tissue site in an animal, comprising contacting the tissue site with the in situ bioreactor of any one of claims 1-18.

73. The method of claim 72, wherein the tissue site is the site of an iatrogenic injury.

74. The method of claim 72, wherein the tissue site is subcutaneous, intramuscular, intraperitoneal, or retroperitoneal.

75. The method of claim 72, wherein the tissue site is an organ.

77. The method of claim 76, wherein the mammal is a human.

79. The method of claim 72, wherein subsequent to contacting the tissue site reactor, the bioreactor is supplemented with additional quantities of the second molecule encoding a bioactive agent.

81. The method of claim 72, wherein prior to contacting the tissue site with the bioreactor, the bioreactor comprises cells transduced with the first nucleic acid, the second nucleic acid, or both the first and second nucleic acids.

83. A method adapted for systemic delivery of a protein from a tissue site, comprising contacting a tissue site of an animal with an in situ bioreactor, wherein the bioreactor

comprises a biocompatible substance and a first nucleic acid molecule, wherein the first nucleic acid molecule encodes a cell growth stimulating agent, and wherein said encoded cell growth stimulating agent conditions matrix infiltrating cells for uptake of a second nucleic acid molecule encoding a serum soluble protein.

84. The method of claim 83, wherein the cells are selected from the group consisting of: stem cells, macrophages, fibroblasts, and vascular cells.

85. The method of any one of claims 82-83, wherein the nucleic acid molecules are absorbed in the biocompatible substance.

86. The method of any one of claims 82-83, wherein the nucleic acid molecules are adsorbed to the biocompatible substance.

87. The method of any one of claims 82-83, wherein the nucleic acid molecules are impregnated within the biocompatible substance.

88. The method of any one of claims 82-83, wherein the biocompatible substance is a mixture of synthetic and biological materials.

89. A method adapted for systemic delivery of a protein from a tissue site, comprising introducing into a tissue site of an animal an in situ bioreactor, wherein the bioreactor comprises a biocompatible substance, a cell growth stimulating agent, and a first nucleic acid molecule encoding a serum soluble protein wherein the bioreactor is capable of infiltration by cells.

90. The method of claim 89, further comprising introducing a second nucleic acid molecule into the bioreactor.

91. The method of claim 90, wherein the second nucleic acid molecule encodes a growth factor.

92. The method of claim 90, wherein the second nucleic acid molecule is introduced subsequent to introduction of the bioreactor into the tissue site.

93. The method of claim 89, wherein the growth stimulating agent is a nucleic acid molecule encoding a growth factor.

94. The method of claim 89, wherein the growth stimulating agent is a protein.

95. The method of claim 94, wherein the protein is a growth factor.

96. The method of claim 89, wherein the protein is an angiogenic factor.

97. The method of claim 89, further comprising the step of seeding the bioreactor with cells prior to introduction to the tissue site.

98. A Bi-gene device comprising a biocompatible substance capable of cellular infiltration, a first nucleic acid molecule encoding a cell growth stimulating agent, and a second nucleic acid molecule encoding a bioactive agent.

99. The device of claim 98, wherein the biocompatible substance is a synthetic substance or biological substance.

100. The device of claim 98, wherein the device is non-biodegradable.

101. The device of claim 98, wherein the biocompatible substance comprises a substance selected from the group consisting of: PTFE, expanded PTFE, Dacron, metal,

polylactic acid, polyglycolic acid, polylactic-polyglycolic acid (PLGA), collagen, bioceramic materials, alginate, and hyaluronic acid.

102. The device of claim 98, wherein the device may be supplemented with additional nucleic acid molecules.

103. A kit for the production of a device, comprising:

- a) an appropriate container,
- b) a biocompatible substance,
- c) a first nucleic acid molecule encoding a cell growth stimulating agent; and
- d) a second nucleic acid molecule encoding a bioactive agent.

104. A kit for the production of a coated device, comprising:

- a) a device coated with a biocompatible substance,
- b) a first nucleic acid molecule encoding a growth stimulating agent; and
- c) a second nucleic acid molecule encoding a bioactive agent.